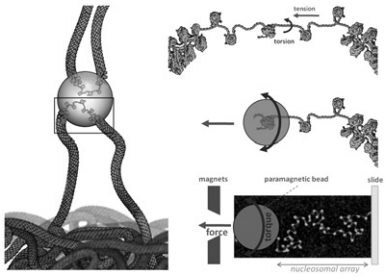
allowed us to extract quantitative kinetic parameters that precisely describe these processes in living cells.
Supported by NIH GMS Grants to RH Singer.

3099-Symp
Into the CoSMoS: Single Molecule Analysis of Spliceosome Assembly and Activation
Aaron A. Hoskins[1], Larry J. Friedman[2], Daniel J. Crawford[3], Eric J. Anderson[3], Inna Shcherbakova[3], Virginia W. Cornish[4], Jeff Gelles[2], **Melissa J. Moore**[5].
[1]Univ. Wisconsin, Madison, WI, USA, [2]Brandeis University, Waltham, MA, USA, [3]UMass Medical School/HHMI, Worcester, MA, USA, [4]Columbia University, New York, NY, USA, [5]Department of Biochemistry and Molecular Pharmacology, UMass Medical School/HHMI, Worcester, MA, USA.
Excision of introns from pre-mRNAs is mediated by the spliceosome, a large, dynamic complex consisting of five small ribonucleoprotein particles (snRNPs) and scores of associated proteins. Current understanding of spliceosome assembly is based largely on the procession of stable complexes that can be resolved from in vitro splicing reactions. Such ensemble experiments have suggested a highly ordered, linear assembly pathway in which initial binding of U1 snRNP to the 5' splice site is followed by stable U2 association with the branch site and subsequent U4/U5/U6 tri-snRNP and Nineteen Complex (NTC) addition to form the fully assembled spliceosome. Previously unknown, however, were the detailed forward and reverse kinetics of each assembly step, the extent to which branched and/or dead-end assembly pathways exist, and whether or not different introns utilize the same or alternate assembly pathway(s). We are now addressing these questions by combining yeast genetic engineering, chemical biology, and multi-wavelengthfluoresence microscopy to follow assembly of single spliceosomes in real time. Because no protein purification or reconstitution is required for such Colocalization Single Molecule Spectroscopy (CoSMoS), this experimental strategy should prove widely useful for mechanistic analysis of many other macromolecular machines in environments approaching the complexity of living cells.

3100-Symp
Chromatin Dynamics: At the Source of RNA Production
Christophe Lavelle.
National Museum of Natural History, Paris, France.
Through its local heterogeneities and transient structural changes resulting from chemical modifications and physical constraints imposed by numerous actors in vivo, chromatin dynamics influences (and is influenced by) DNA transcription, both at the initiation and elongation stages.
While transcription events often correlate with large chromatin movements, RNA polymerase access to its target sequence implies some nucleosome dynamics (potentially mediated by chromatin remodeling factors) and its subsequent tracking along the DNA template imposes some drastic topological and structural changes that propagate in the transcribed chromatin domain. Experiments and models aimed at deciphering the key features of chromatin dynamics and topology upon transcription will be presented.



In a second part of the talk, we will shift from eukaryotes to prokaryotes and show some recent data on ncRNA self-assembly, which role (and potential occurrence in eukaryotes) will be questioned.

3101-Symp
Biophysics of the Interaction of HIV with Mucus Barriers during Sexual Transmission
Thomas Hope.
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During sexual transmission, HIV must overcome mucosal barriers to reach underlying target cells. The epithelial barrier function of the female reproductive tract is further enhanced by a protective layer of cervical mucus (CM). It is believed that antibodies associated with mucus barriers of the gut can have antimicrobial activity, and so we explored the possible role that antibodies which can bind to virions might alter particle transport through mucus. To characterize the effects of HIV-specific antibodies on viral diffusion in CM, we utilized two red and green fluorescently tagged HIV types: wildtype enveloped virus (WT-Gag-mCherry) and HIV devoid of envelope proteins, (ΔEnv-Gag-GFP) which allows for simultaneous visualization of both virus types in CM. For our assay, both virus types were mixed at equal concentrations and either added directly to CM or incubated with HIV-specific antibodies prior to being added to CM. Particle tracking software was used to determine particle position and measure the mean squared displacement (MSD), a standard measure of microscopic motion. We found that using anti-MHC class I antibodies, which bind epitopes found on WT and ΔEnv virus, decrease the movement of both virus types. In addition, neutralizing and non-neutralizing anti-gp41 and anti-gp120 antibodies specifically impaired the mobility of WT virus when compared to ΔEnv virus or when compared to virions that received no antibody treatment. In addition, we observed greater inhibition of HIV transport in CM with multimeric anti-Env antibodies when compared to monomeric antibodies. Our studies reveal that virus binding antibodies can slow the transport of HIV within the mucus coating of the female reproductive tract. This suggests that a vaccine that generates broadly binding antibodies could potentially prevent viral interactions with target cells thus offering protection against productive infection.

Minisymposium: Molecular Motors: Stopped or Slowed by Their Tracks

3102-MiniSymp
Single-Molecule Protein Unfolding and Translocation by the AAA+ Protease, ClpXP
Marie-Eve Aubin-Tam[1], Adrian O. Olivares[1], Juan Carlos Cordova[2], Stephane Calmat[1], Robert T. Sauer[1], Tania A. Baker[1], Matthew J. Lang[2].
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All cells employ ATP-fueled AAA+ proteases for protein-quality control and regulation. In the ClpXP protease, the ring-shaped AAA+ molecular motor ClpX first recognizes and mechanically unfolds specific protein substrates, then translocates the denatured polypeptide through a central axial pore into the barrel-shaped peptidase ClpP for degradation. Although the mechanisms of ATP utilization and substrate degradation have been extensively studied in solution and recently at the single-molecule level[1, 2], the molecular details underlying mechanical substrate unfolding and stepping along the polypeptide track remain unexamined.
Using a dual-laser optical trap, we probed how ClpXP unfolds and translocates a multi-domain protein substrate at the single-molecule level. In our assay, a ClpXP-substrate complex is tethered between two trapped polystyrene beads held in a dumbbell configuration [2]. Motility records of ClpXP along the polypeptide track show unique, fingerprint-like, substrate unfolding and translocation events. Following rapid and cooperative unfolding of individual domains, we find that ClpX translocates the polypeptide into ClpP, taking small steps of 5-8 amino acids. The nature of the polypeptide track affects ClpXP mechanochemical activity. ClpX step size does not depend on ClpP, though we observe substantial substrate refolding and slippage events when only ClpX is examined. Our results support a power-stroke model of denaturation in which successful unfolding requires mechanical pulling by the enzyme to coincide with transient stochastic protein destabilization.
[1] Shin, Y., Davis, J.H., Brau, R.R., Martin, A., Kenniston, J.A., Baker T.A., Sauer, R.T., Lang, M.J. Single-molecule denaturation and degradation of proteins by the AAA+ ClpXP protease. PNAS, 106, 19340 (2009)
[2] Aubin-Tam, M. E., Olivares, A. O., Sauer, R. T., Baker, T. A., Lang, M. J. Single-molecule protein unfolding and translocation by an ATP-fueled proteolytic machine. Cell, 145, 257-67 (2011)

3103-MiniSymp
Single-Molecule Imaging Reveals Mechanisms of Roadblock Clearance by DNA Motor Enzymes
Ilya J. Finkelstein[1], Ja Yil Lee[1], Estelle Crozat[2], David J. Sherratt[3], Eric C. Greene[1].
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In the cell, nucleic acid motor proteins act on substrates occupied by other proteins, yet little is known regarding the inevitable collisions that must occur. Using nanofabricated curtains of DNA and real-time, multi-color single-molecule microscopy we visualized collisions between two model translocases and DNA-bound obstacles. We show that both RecBCD, a